Intralymphatic Immunotherapy: Update and Unmet Needs

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Keywords
Allergen-specific immunotherapy · Allergy · Clinical trials · Intralymphatic immunotherapy

Abstract
Allergen-specific immunotherapy (AIT) is the only allergy treatment that confers long-term symptom amelioration for patients suffering from allergy. The most frequently used allergen application route is subcutaneous injection (SCIT), commonly taken as the gold standard, followed by sublingual (SLIT) or oral (OIT) application of allergen preparations. This is an up-to-date review of the clinical evidence for a novel route of allergen application, i.e., directly into lymph nodes – intralymphatic immunotherapy (ILIT). The major advantages of ILIT over the current AIT approaches are its short duration and the low allergen doses administered. The whole treatment consists of merely 3 ultrasound-guided injections into inguinal lymph nodes 1 month apart. While the number of patients included in randomised controlled trials is still limited, the clinical results for ILIT are encouraging, but more clinical trials are needed, as well as more preclinical work for optimising formulations.

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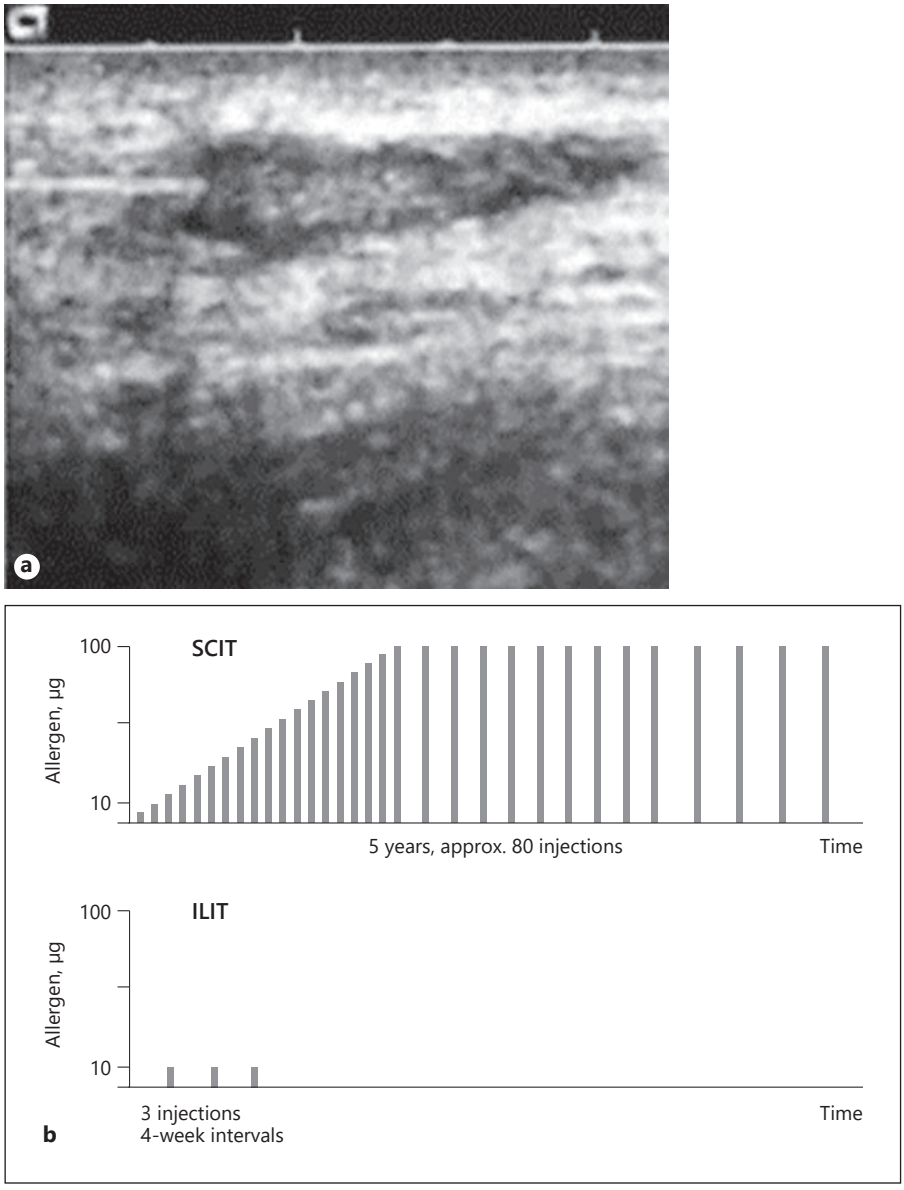

Fig. 1. a Sonographic view of a needle positioned in an inguinal lymph node. **b** Schematic view of the injection protocol for SCIT and ILIT.

ILIT Updated

Introduction

Allergen-specific immunotherapy (AIT) is the only allergy treatment that confers long-term symptom amelioration. AIT administers gradually increasing allergen doses to the allergic individual, thereby altering the immune response to the allergen. The most frequently used allergen application route is subcutaneous injection, commonly taken as the gold standard, followed by sublingual or oral application. Epicutaneous and intralymphatic (ILIT) routes of application are currently in clinical development. Also, intradermal [1] and local nasal [2] routes have been explored. AIT has recently been reviewed [3, 4].

The effectiveness and the efficacy of the diverse approaches in AIT depend largely on the composition and formulation of the applied allergen, recently reviewed by Akdis and Akdis [5], as well as on adjuvants, recently reviewed by Chesné et al. [6]. An all-encompassing review was scheduled for 2016 by the European Academy of Allergy and Clinical Immunology (EAACI) [7].

A PubMed search for controlled clinical trials in early 2016 yielded more than 120 publications on subcutane-

ous immunotherapy (SCIT), over 300 on sublingual immunotherapy (SLIT) and oral immunotherapy (OIT), 17 on local nasal immunotherapy, 5 on epicutaneous immunotherapy (EPIT), 4 on intralymphatic immunotherapy (ILIT), and 1 on intradermal immunotherapy (with peptides for Treg induction). With the exception of SLIT, most clinical trials were conducted with relatively small numbers of patients, and with a diversity of allergens, adjuvants, and formulations.

The scarcity of investigations into novel routes may at least in part be due to new regulations which currently force the industry to officially register their therapeutic allergen extracts. Thus, the industry is currently focussed on efficacy trial programs to keep existing products on the market. Another reason for the lack of commercial interest may be that the newer methods such as ILIT tend to use much less allergen. On the other hand, a recent major production bottleneck of one major supplier of immunotherapeutic vaccines has compromised treatment cycles of SCIT in Europe [8], highlighting an advantage of immunotherapy methods with fewer and lower-dose vaccine applications that may be less affected by a compromised supply chain.

ILIT is one such application route requiring merely 3 ultrasound-guided injections of low allergen doses into inguinal lymph nodes with a 1-month time interval (Fig. 1). The entire treatment is therefore finished after 2 months. In comparison, SCIT with common marketed allergen extracts requires up to 70 injections and visits to a medical practice over a time period of up to 5 years, something that only 5% of eligible patients judge worth the inconvenience [9]. ILIT poses a viable alternative to overcome the most urgent problem with long-term SCIT and SLIT management, oftentimes overlooked by the medical community, i.e., non-adherence to treatment [10]. ILIT considerably reduces the resource overhead for the patient, the physician, and the reimbursing party. Finally, the relatively short treatment interval avoids using multiple batches of vaccine or treatment interruptions due to supply chain bottlenecks. If a single batch of vaccine is not in stock for a whole treatment cycle, the immunotherapy will not be started until supply is resumed, thus avoiding interruptions, premature terminations, or adverse reactions due to change of batches during the course of AIT that might lead to an unsatisfactory outcome.

ILIT Clinical Trials

In Table 1 we present all clinical trials ($n = 8$) published to date on intralymphatic allergen immunotherapy, including 175 patients receiving intralymphatic injections of verum and 83 and 54 receiving placebo or SCIT, respectively. In 2015, Senti et al. [11] reviewed the accumulated experience in ILIT up to 2014. They reviewed all 4 clinical trials on ILIT in humans published until mid-2014 [12–15]. Their conclusion from the 4 trials was that they indicate ILIT against grass pollen and bee venom to not only be safe and efficient, but also associated with a lower risk of systemic adverse effects (e.g., anaphylaxis and lethal consequences). With only 3 injections in 3 months, symptom relief was documented as comparable with standard SCIT requiring up to 100 injections over 3–5 years. The immunological data of one of these trials, in which the major cat dander allergen was fused with a molecular antigen transportation system (MAT-Fel d 1), has meanwhile been worked up in detail by Zaleska et al. [16].

In 2016, Hylander et al. [9] reported a double-blinded placebo-controlled clinical trial in 36 patients with birch- or grass pollen-induced rhinoconjunctivitis and concluded that ILIT is an effective and safe therapy for this condition, resulting in a marked reduction of seasonal allergic symptoms. In the same year Patterson et al. [17] published a randomised, double-blind, placebo-controlled, parallel group pilot study in 15 adolescents in the USA with bothersome nasal, ocular, and/or respiratory symptoms correlating with the timing of the grass pollen season and evidence of grass pollen sensitisation. They concluded ILIT to be efficacious and safe, and recorded notably low adverse reactions.

All ILIT trials so far have noted a clinical improvement except for the one reported by Witten et al. [15] that showed immunological changes but no clinical improvement. If anything, symptoms tended to worsen. In contrast to all other successful trials, the interval between injections in that trial by Witten et al. [15] was shorter than in other trials, i.e., 2 instead of 4 weeks. This may explain the missing clinical effect as time intervals shorter than 2 weeks are known to compromise memory B cell formation and affinity maturation [18, 19]. It is well known in vaccine immunology that both these processes require periods where antigen levels are low, so that there is competition for the antigen in germinal centres that can positively select high-affinity B cells [18].

An open label pilot study with 7 patients with grass pollen-related seasonal rhinoconjunctivitis showed an induction of allergen-specific plasmablasts expressing other isotypes than IgE and a trend towards improvement of symptoms, medication score, and rhinoconjunctivitis-related quality of life during one pollen season

Int Arch Allergy Immunol
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Table 1. Publications of ILIT clinical trials (up to 2018)

Trial	Trial No. (ClinicalTrials.gov)	Trial type	Allergy	Vaccine	Interval	Placebo	Patients with verum, *n*	Patients with placebo, *n*	Patients with reference treatment, *n*	Follow-up	Endpoints (primary in bold)	Statistical method	Ref. No.
A randomised study to evaluate a novel method of specific allergen immunotherapy	NCT00470457	Monocentric open-label randomised controlled	Grass pollen	Aluminium hydroxide-adsorbed grass pollen extract (Alutard SQ; ALK-Abellò)	4 weeks	NA	58	NA	54	October–October: 144 weeks	**Seasonal allergic symptoms by visual analogue scale**. Adverse events. Safety of injections. Use of rescue medication. Skin-prick test. Grass-specific IgE levels	Mann-Whitney U test. χ^2 test; general linear model repeated-measures analysis. Student *t* test; Wilcoxon signed-rank test. Friedman test	14
Evaluation of safety, tolerability, immunogenicity and efficacy of a novel method in specific immunotherapy in cat allergic patients: a placebo-controlled trial	NCT00718679	Randomised double-blind placebo-controlled	Cat dander	Aluminium hydroxide (Alhydrogel 2%; Brenntag Biosector, Frederikssund, Denmark) adsorbed MAT-Fel d 1, a recombinant non-glycosylated fusion protein consisting of 291 amino acids (manufactured by Strathmann Biotec GmbH & Co. KG, Hamburg, Germany)	4 weeks ±3 days	Aluminium hydroxide (Alhydrogel 2%; Brenntag Biosector, Frederikssund, Denmark) in saline	12	8	NA	Approx. 55 weeks	**Titrated nasal provocation test**. Immunological parameters. Systemic adverse effects. Skin-prick test. Validated rhinitis quality-of-life questionnaire	Wilcoxon signed-rank test; Wilcoxon test. Welch *t* test; Pearson correlation coefficient	13
Study to assess efficacy of intralymphatic immunotherapy	NCT01166269	Randomised, double-blind, placebo-controlled	Grass pollen	Depot grass pollen extract (Alutard, Phleum pratense, ALK-Abellò)	≥2 weeks	Saline	29	14	NA	June–August: 13 weeks	**Combined symptom and medication score (SMS)**. Global seasonal assessment; rhinoconjunctivitis quality of life questionnaire (RQLQ)	ANOVA with Tukey correction; *t* test	15
Intralymphatic immunotherapy in increasing doses, after subcutaneous immunotherapy	NCT02679105	Open-label pilot + randomised double-blind placebo-controlled	Birch/ grass pollen	Aluminium hydroxide-adsorbed, depot birch pollen or grass pollen (Alutard; ALK-Abellò)	4 weeks	Allergen diluents without aluminium hydroxide (ALK-Abellò)	7	8	7 SCIT, birch pollen (Alutard; ALK-Abellò)	Spring–Autumn: approx. 24 weeks	**Seasonal allergic symptoms** by visual analogue scale and **medications score**. Skin-prick test. Nasal provocation test. Nasal lavage fluid. Immunological parameters	D'Agostino and Pearson omnibus normality test; paired *t* test. Wilcoxon matched-pairs signed rank test; Mann-Whitney U test. Fisher exact test	12
A randomised study to evaluate a novel method of specific allergen immunotherapy	NCT00470457	Same as in [14]	Grass pollen	Same as in [14]	4 weeks	NA	Same patients as in [14]	NA	Same patients as in [14]	Same as in [14]	**Seasonal allergic symptoms** by visual analogue scale after **titrated nasal provocation test (tNPT)**	Mann-Whitney U test; maximally selected test statistics. Hierarchical ordered logistic model	26
Evaluation of safety, tolerability, immunogenicity and efficacy of a novel method in specific immunotherapy in cat-allergic patients: a placebo-controlled trial	NCT00718679	Same as in [13]	Cat dander	Same as in [13]	4 weeks ±3 days	Same as in [13]	Same patients as in [13]	Same patients as in [13]	NA	Same as in [13]	**Safety tolerability and efficacy.** Specific T cell proliferation, IL-10- and IFN-γ-dominated T cell response, Th2 cytokines, allergen-specific Treg cells, circulating Fel d 1 tetramer-positive cells, IL-10 and FOXP3 expression, and in HR2/HR1	Wilcoxon signed-rank test, Mann-Whitney U test, ANOVA. Dunn's multiple comparison test correction	16

Table 1 (continued)

Trial	Trial No. (ClinicalTrials.gov)	Trial type	Allergy	Vaccine	Interval	Placebo	Patients with verum, *n*	Patients with placebo, *n*	Patients with reference treatment, *n*	Follow-up	Endpoints (primary in bold)	Statistical method	Ref. No.
Safety of lymph node injection for allergen immunotherapy	NCT01982474	Randomised, double-blind, placebo-controlled, parallel group pilot	Grass pollen	Aluminium hydroxide-adsorbed grass pollen extract Center-Al *Phleum pratense* (ALK, Round Rock, TX, USA)	4 weeks	Saline with phenol	7	8	NA	May–July: 10 weeks	**Severe adverse events.** Local and systemic symptom score after injections (total safety score, TSS); patient diary score of allergy and asthma symptoms and medication use (total combined score, TCS)	TSS numeric summaries; Mann-Whitney U test using ranks from the aggregate score and each injection individually	17
Safety and efficacy study of intralymphatic allergen-specific immunotherapy	NCT02423707	Parallel double-blind placebo-controlled	Birch/ grass pollen	Standardised, aluminium hydroxide adsorbed, depot birch- or grass-pollen vaccine (Alutard, ALK-Abellò)	3–4 weeks	Alutard (ALK-Abellò, Horsholm, Denmark)	21, including 7 of [12]	15, including 8 of [12]	NA	January–September: 36 weeks	**Seasonal allergic symptoms** by visual analogue scale, nasal symptom score, NSS, following nasal provocation test, NPT; safety of injections; circulating IgE and IgG4 levels. Change in inflammatory cells. Use of rescue medication	Student unpaired *t* test; repeated measures ANOVA followed by Dunnet's multiple comparison post-test; Friedman test, followed by Dunn's multiple comparison post-test; two-way ANOVA, followed by the uncorrected Fisher's LSD test; Pearson's correlation coefficient	9
Open-labelled pilot study of intralymphatic immunotherapy (ILIT) for house dust mite, cat and dog allergen in allergic rhinitis patients	NCT02301884	Open-label, single group assignment	House dust mite, dog and cat	Aqueous allergen extracts (HollisterStier, New Orleans, LA, USA)	4 weeks	None	11	0	NA	42 weeks	Clinical efficacy and safety; **rhinoconjunctivitis quality of life questionnaire**, nasal provocation test; skin-prick test, use of rescue medication. Visual analogue scale for pain, sino-nasal outcome test-20	Paired Mann-Whitney U test, Fisher's exact test	21
A randomised placebo-controlled study using combined grass- and tree pollen intralymphatic immunotherapy; one allergen given on each side	NCT02423707	Randomised double-blind placebo controlled	Birch and grass pollen	Aluminium hydroxide-adsorbed, depot birch pollen or grass pollen (Alutard; ALK-Abellò)	4 weeks	Allergen diluents without aluminium hydroxide (ALK-Abellò)	24	27	NA	6–9 months	**Nasal provocation test**, VAS for overall improvement, rhinitis quality of life questionnaires, medication use, skin-prick tests	Friedman, Kruskal-Wallis, Mann-Whitney, Fisher's exact tests, χ^2 tests, Wilcoxon matched-pairs signed-rank tests	22

Two publications refer to a trial reported before [16, 26] and one trial was an extension of a previous one [12]. NA, not available.

Int Arch Allergy Immunol
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[20]. Another open label pilot study with 11 patients suffering from rhinoconjunctivitis caused by house dust mite, cat or dog allergens, or a combination thereof, indicated a risk of systemic adverse effects after ILIT but also found that ILIT can rapidly improve allergic rhinitis symptoms and reduce the frequency of prescription of rescue medication, and that the effect lasted for 1 year [21].

The only long-term study published to date [14] showed a sustained clinical benefit (up to 3 years) in terms of symptom reduction, comparable to SCIT. One trial with 60 patients randomised 1:1 to receive combined injections of birch and grass pollen allergen or placebo found the treatment to be safe and effective [22]. The sum of nasal symptoms was reduced by 25% at follow-up after 6–9 months in the verum group, whereas no reduction was observed in the placebo group. The authors also found as a first-time discovery mirrored in the blood an increased proportion of effector memory T cells in lymph node-derived cells.

Endpoints

Clinical trials in AIT have subjective patient-related outcomes and objective read-outs. A recent EAACI position paper concluded that the most recommended subjective scoring is the combined symptom-medication score [23]. Quality of life, challenge testing, and immunological outcomes can be used as secondary efficacy parameters. As listed in Table 1, all ILIT clinical trials have used subjective symptoms recorded by patients as an endpoint. One trial used a combined score of symptoms and rescue medication as an endpoint. Various scores or visual analogue scales were used to quantify the symptoms in other studies. Use of rescue medication was an endpoint in 3 trials. Two trials used a rhinoconjunctivitis quality of life questionnaire, the original "RQLQ" [24] and the validated "mini-RQLQ" [25], respectively. Further endpoints were: (i) nasal symptoms after a nasal provocation test in 3 trials, (ii) immunological parameters (circulating IgE and/or IgG4 levels, and T cell analyses) in 3 trials, (iii) skin-prick test in 3 trials, and (iv) nasal lavage fluid parameters in 1 trial. All trials also recorded adverse events according to regulations and local reactions at the injection site as safety endpoints.

There is no consistency in the choice of endpoints except subjective symptom scores. For the assessment of symptoms, a diversity of non-standard and non-validated scoring methods were used, except for 3 trials using RQLQ.

Allergen Extracts Used

Table 1 shows that in 5 ILIT clinical trials with patients suffering from seasonal grass and/or birch allergic rhinitis or rhino-conjunctivitis (ARC), commercially available grass pollen extract was injected, and in 2 of these trials commercially available birch pollen extract was used in the ARC patients with birch allergy. One trial used a recombinant MAT-Fel d 1 vaccine produced in a GMP facility in patients suffering from cat dander ARC [13]. All but one [21] of the ILIT clinical trials used aluminium hydroxide-adsorbed antigen as vaccines. None of the ILIT trials used any additional adjuvant.

The first ILIT open-label clinical trial [14] was re-analysed for methodological effects in measuring and analysing the efficacy of immunotherapies of grass pollen allergy [26]. The conclusions included a need for methodological recommendations, as the choice of analytical methods affects the outcome.

One pilot study [21] also used combinations of allergen extracts according to the hypersensitivity pattern of the patient. One trial used a combined injection of birch and grass pollen allergen [22].

Immunology of ILIT

More than 20 years ago, Kündig et al. [27] proposed a simple geographical model of immunogenicity, i.e., that antigens in the periphery are largely ignored, whereas antigens which reach a lymph node, either by being drained there or by being transported there by dendritic cells, are strongly immunogenic. One important reason for the fact that a lymph node is such an immunogenic environment is the high probability for an antigen to meet a specific T or B cell, which is orders of magnitude higher than with peripherally administered antigens, because of the enormous density of T and B cells. ILIT has proved to enhance the immune response when using various types of vaccines, i.e., proteins, peptides, mRNA, naked DNA, bacteria, immunostimulatory complexes (ISCOMS), virus-like particles, and dendritic cells. Also, ILIT has been successfully performed in various animal species, such as mice, rats, dogs, horses, ponies, cows, and monkeys, as reviewed previously [28]. When analysing human sera after ILIT with MAT-Fel d 1, Freiberger et al. [29] recently found that the predominant immunoglobulin subclass was IgG4, whereas only marginal levels of IgG1, IgG2, and IgG3 were induced. We previously showed that this predominant IgG4 induction correlated with the allergen-specific IL-10-producing T cell response [13]. Further analyses confirmed that early allergen-specific T cell activation by ILIT was followed by T cell un-

responsiveness to allergen, characterised by increased allergen-specific IL-10-producing Treg cells expressing FOXP3 [16].

ILIT and Unmet Needs

Only a few trials reported systemic adverse reactions after intralymphatic administration of antigen. As the needle penetrates all skin layers before reaching the lymph node it is possible that antigen solution leaks from the needle tip into those layers or even into inadvertently penetrated vessels. The ultrasound-guided targeting of the lymph node may not be precise enough in every case to exclude the possibility of inadvertently injecting antigen into surrounding subcutaneous tissue. Both situations should only rarely occur in the hands of adequately trained personnel. The requirement of ultrasound equipment may be considered a drawback of this therapeutic approach, and the inguinal site of injection may not be appealing to some patients.

Despite the predominantly favourable reports of clinical trials with ILIT and the observed immunologic changes that together can be taken as proof of concept, there is as yet not enough convincing evidence for a routine use of ILIT in treating allergic conditions, nor is there any authorised product for ILIT commercially available in any country. The most pressing unmet need is not only for higher numbers of patients/controls and more multicentric prospective randomised controlled double-blinded clinical trials, but also for the use of more diverse allergens. While grass or birch pollen and cat dander allergies are among the most frequent allergies that are not easily avoidable, worldwide the most important allergen is house dust mite. There are many more seasonal and perennial allergic conditions waiting for shortened AIT regimes. Also, a shorter and effective immunotherapy for insect venom hypersensitivity would be most welcome for affected patients, as the frequency of adverse events with the rush-build-up schedule of venom immunotherapy is still considerable. More bench work followed by clinical testing is also needed for optimising the vaccines. The vaccines tested so far were all adsorbed to aluminium hydroxide with no other and more potent biological response modifiers added. There is a good deal of know-how in the field of immunisation that could be applied to ILIT. Another major unmet need that is not specific to ILIT or AIT in general is the harmonisation of inclusion/exclusion criteria, endpoints, and detection/monitoring methods in allergy trials. Whereas there is a consensus in choosing subjective symptoms as a major endpoint, there are no common procedures in quantifying or assessing symptoms, or for including the effect of medication usage on symptom scores. The same is true for many "objective" endpoints, where there is a diversity in analytical methods, validated or not, with untrusted reference ranges. All this hampers the statistical power of any single trial and robustness of meta analyses. Table 2 lists common items that need to be agreed on in order to maximise the scientific and clinical gain of knowledge with the limited financial resources available for clinical trials and basic research. Previously published guidelines recommending how to standardise AIT trials [30] and AIT trial outcomes [23] could be tailored to the specific needs of ILIT trials. ILIT continues to be underexplored in paediat-

Table 2. Open questions or missing consensus, common to ILIT and other AIT

Questions/problems
Patient eligibility
Mono-allergic
Poly-allergic
Minimal age
Comorbidities
Administration schedule
Number of injections
Interval
Pre-/co-/post-seasonal
Dosage
Amount
Volume
Concentration
Constant
Escalating
Standardisation
Biological
Immunological
Reference
Vaccine
Choice
Production
Mix
Adjuvants
Choice
Dose/concentration
Outcome measuring
Symptoms/quality of life
Functional tests
Laboratory parameters
Study design
Mono-/multicentric
Open label/blinded
Prospective/retrospective
Registry

ric patients and further investigation in this population is mandatory to establish adequate clinical guidelines of its indications and outcomes. It may be worth mentioning that ILIT has recently been successfully tested for the treatment of canine atopic dermatitis [31].

Conclusion

With the 6 clinical trials reported so far with: (i) a total of 134 patients receiving intralymphatic injections of verum and 53 and 54 receiving placebo or SCIT, respectively, (ii) inconsistent endpoints with non-uniform methods of assessing endpoints, (iii) often small difference (delta), and (iv) inconsistent and mainly short follow-ups, the overall statistical power is obviously moderate. Nonetheless, all but 1 study reported desirable measurable effects compared to controls (placebo in 5, SCIT in 1 trial). This effect of the intervention observed across the studies and consistently pointing in the same direction is likely to support the findings of Atkins et al. [32]. Only 1 study reported an effect on immunological parameters, but no symptomatic improvement compared to placebo [15]. It is worth noting that the only long-term study published to date [14] showed sustained symptom reduction for up to 3 years, representing a clinical benefit comparable to SCIT. Although this evidence needs to be confirmed by further studies, this may suggest that a persistent effect may be obtained by a short course of ILIT. While it is still too early to recommend ILIT for clinical use, proof of principle is established. Unanimously, the trials found ILIT to be safe. With only 3 injections with 1-month time intervals, and therefore a treatment duration of merely 2 months, the inconvenience for the patient and the overhead for the clinical practice is considerably lower than with up to 70 practice visits for injections over up to 5 years for the gold standard SCIT, thus enhancing treatment adherence. The health economic impact [33, 34] of ILIT may be substantial due to reduced consumption of resources during treatment, but also, when successful, due to reduced use of rescue medication for symptomatic treatment and fewer sick leaves.

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None of the authors have any competing interests to declare.

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